Oxidative Stress Change by Systemic Corticosteroid Treatment Among Patients Having Active Graves Ophthalmopathy

Chieh-Chih Tsai, MD; Shu-Ching Kao, MD; Ching-Yu Cheng, MD, MPH; Hui-Chuan Kau, MD, MS; Wen-Ming Hsu, MD; Cheng-Feng Lee, PhD; Yau-Huei Wei, PhD

Objective: To measure the 8-hydroxy-2′-deoxyguanosine (8-OHdG) level in patients having active Graves ophthalmopathy (GO) and to compare this oxidative stress biomarker and the clinical evolution of patients after systemic corticosteroid treatment.

Methods: In 8 euthyroid patients having active GO, we determined the 8-OHdG levels in urine before, during, and after intensive corticosteroid therapy. Clinical activity and ophthalmopathy index scores were assessed. Nine age- and sex-matched healthy volunteers served as control subjects.

Results: The mean 8-OHdG level was statistically significantly increased in patients having active GO compared with that of controls (17.47 vs 5.97 ng/mg of creatinine, P < .001). During and after maximal systemic corticosteroid treatment, patients had statistically significantly lower mean 8-OHdG levels (7.19 and 10.18 ng/mg of creatinine, respectively) compared with the mean level before treatment. These changes were accompanied by decreases in clinical activity and ophthalmopathy index scores. The urinary 8-OHdG levels were subsequently elevated in 2 patients having recurrent active GO when corticosteroid therapy was tapered or withdrawn.

Conclusions: Oxidative stress may have a role in the pathogenesis of GO. Urinary 8-OHdG level can be used not only as a noninvasive biomarker of oxidative stress in patients having GO but also as an objective and quantitative parameter in the follow-up of patients during immunosuppressive treatment.

Arch Ophthalmol. 2007;125(12):1652-1656
In this study, we measured the 8-OHdG levels in urine of patients having active GO treated with systemic corticosteroids with an aim to determine whether 8-OHdG level could be used as a biomarker in the management of GO.

**METHODS**

**PATIENTS**

Between June 1, 2006, and May 31, 2007, all patients who had developed ocular signs during the past 6 months and demonstrated active GO with a clinical activity score (CAS) of 4 or higher at the beginning of therapy and who underwent systemic corticosteroid therapy at Taipei Veterans General Hospital were prospectively recruited for the study. Before inclusion in the study, euthyroidism in all patients having GO was achieved by medication (antithyroid drugs only, including carbimazole, methimazole, and propylthiouracil, or combined with thyr-oxine sodium) for at least 6 months. None were treated with radioactive iodine. The diagnosis of GO was based on the criteria proposed by Bartley and Gorman. Computed tomography of the orbit and Hertel exophthalmometry were performed to help confirm the diagnosis of GO. Control subjects were recruited from age-matched and sex-matched healthy persons who were enrolled when they attended their routine physical examinations. Exclusion criteria were pregnancy, alcohol drinking, any ocular diseases other than GO, regular drug ingestion or antioxidant use, and GO with a history of surgical decompression, systemic corticosteroid therapy, or radiation therapy, as well as individuals with chronic or acute disease such as cancer, hypertension, hyperlipidemia, diabetes mellitus, and diseases of the lung, liver, or kidney or other endocrine, immunologic, or inflammatory disorders. The study was approved by the institutional review board of Taipei Veterans General Hospital, and patients gave informed consent for their participation.

**TREATMENT, FOLLOW-UP, AND URINE COLLECTION**

In the GO group, systemic corticosteroid therapy was oral prednisolone (0.75 mg/kg of body weight daily) administered for 4 weeks and then tapered slowly to discontinuation at approximately 3 months. All patients underwent ophthalmologic investigation, including assessment of clinical activity and severity of GO and collection of urine before, during (4 weeks after treatment initiation), and after intensive corticosteroid treatment. Graves ophthalmopathy activity was scored according to the CAS suggested by Mourits et al., ranging from 0 to 10 points. Severity of GO was scored using the ophthalmopathy index (OI), based on the NOSPECS mnemonic (composed of the first character describing each grade) classification ranging from 0 to 15 points (0-3 points were given for each ocular change based on the severity of sight loss, proptosis, and soft-tissue, extraocular muscle, and corneal involvement). The definition of proptosis was adjusted according to racial/ethnic variation, and the mean ± SD exophthalmos value among Taiwanese healthy adults is 13.91 ± 2.33 mm. Urine samples were also obtained from the control subjects. Smokers were requested to abstain from smoking overnight before urine collection.

**DETERMINATION OF 8-OHdG LEVEL IN URINE**

Urine specimens were centrifuged at 4000g for 10 minutes, and the supernatant was stored at −70°C until the enzyme-linked immunosorbent assay (ELISA) analysis. The amount of 8-OHdG in urine was measured using an ELISA kit (8-OHdG check; Japan Institute for the Control of Aging, Fukuroi, Japan). Assays were performed according to the manufacturer’s instructions. The specificity of the assay has been established, and the detection range was 0.5 to 200 ng/mL. The urinary 8-OHdG level in each subject was corrected by the creatinine level in urine and is expressed in nanograms per milligram of creatinine.

**STATISTICAL ANALYSIS**

Statistical analysis was performed using commercially available software (STATA; StataCorp, College Station, Texas). Data obtained are expressed as mean ± SD. Normality of these data was assessed using the Shapiro-Wilk test. Comparisons of 8-OHdG levels between patients having GO and the control group were performed using the unpaired t test, and the effect of treatment in the GO group was evaluated using the paired t test. P < .05 was considered statistically significant.

**RESULTS**

Nine patients met the inclusion criteria; however, 1 patient withdrew because of intolerance of oral prednisolone. Data obtained from 8 patients (Table 1) and 9 control subjects (Table 2) were analyzed. No statistically
significant differences in age (P=.90), sex (P>.99), or smoking status (P>.99) were observed between the groups.

In patients having active GO before therapy, the mean urinary 8-OHdG level (17.47 ng/mg of creatinine) was statistically significantly higher than that of controls (5.97 ng/mg of creatinine) (P<.001). In the GO group, the changes in urinary 8-OHdG level, CAS, and OI after treatment are given in Table 3. The mean 8-OHdG level in urine was statistically significantly decreased among patients during maximal systemic corticosteroid treatment (7.19 ng/mg of creatinine, P=.002) and after completion of treatment (10.18 ng/mg of creatinine, P=.01) compared with that before therapy (Figure). However, there was no statistically significant difference in the mean 8-OHdG level among the patients during maximal systemic corticosteroid therapy vs after treatment (P=.07). The mean CAS of patients statistically significantly decreased from 4.88 before therapy to 2.25 (P<.001) during maximal systemic corticosteroid treatment and to 1.86 (P<.001) after completion of treatment. The mean OI of patients statistically significantly decreased from 5.50 before therapy to 3.00 (P=.002) during maximal systemic corticosteroid treatment and to 2.43 (P<.001) after completion of treatment. Recurrence of active GO was noted in patient 3 (Table 1) when prednisolone therapy was tapered and in patient 4 when the drug was withdrawn for 1 month. The urinary 8-OHdG levels were elevated to 17.31 and 14.35 ng/mg of creatinine in patients 3 and 4, respectively. Both patients subsequently received further intravenous corticosteroid therapy and combined orbital radiation therapy.

Oxidative stress has been associated with the development of different degenerative,22 neoplastic,23 and inflammatory24 diseases. As a reliable biomarker of oxidative DNA damage, higher 8-OHdG levels have been observed in various ocular diseases such as trabecular meshwork in patients having glaucoma,25 Eales disease,26 and pterygium tissue.27 To our knowledge, this is the first study to explore increased 8-OHdG levels in urine of patients having active GO and to disclose the alleviation of this biomarker of oxidative DNA damage after treatment with systemic corticosteroids.

Graves ophthalmopathy, as a primary feature of Graves disease, is a disfiguring and potentially sight-threatening disorder that profoundly affects the person’s quality of life. Although several new treatments have been proposed, progress in the management of GO has been slow. Early intervention with novel therapy requires vast knowledge of the disease mechanisms. Many attempts have been made to understand the pathophysiology and pathogenesis of GO;28-31 but the findings are unclear. Graves ophthalmopathy may be caused by a complex interplay of endogenous and environmental factors, which may be associated with ROS. In vitro findings suggest that interleukin 1β, an important cytokine, participates in the autoimmune response of GO and causes an increase in oxygen-free radical production by orbital fibroblasts, as well as that superoxide dismutase and catalase could partially block the accumulation of glycosaminoglycans induced by this cytokine.32 In addition, in patients having GO, superoxide anions have been shown to stimulate proliferation of orbital fibroblasts, which could be inhibited by methimazole or nicotinamide.33,34 In an in vivo study,35 extracellular indexes of ROS metabolism in blood were also found to increase in patients having infiltrative GO. In the present study, we further revealed increased oxidative DNA damage in patients having active GO compared with controls. To eliminate the effect of abnormal levels of thy-

### Table 2. Smoking Status and Urinary 8-Hydroxy-2'-Deoxyguanosine (8-OHdG) Levels Among Control Subjects

<table>
<thead>
<tr>
<th>Control Subject No./Sex/Age, y</th>
<th>Current Smoker</th>
<th>Urinary 8-OHdG Level, ng/mg of Creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/54</td>
<td>Yes</td>
<td>4.50</td>
</tr>
<tr>
<td>2/F/26</td>
<td>No</td>
<td>7.21</td>
</tr>
<tr>
<td>3/F/47</td>
<td>No</td>
<td>5.63</td>
</tr>
<tr>
<td>4/F/43</td>
<td>No</td>
<td>9.12</td>
</tr>
<tr>
<td>5/F/54</td>
<td>No</td>
<td>5.98</td>
</tr>
<tr>
<td>6/F/41</td>
<td>No</td>
<td>4.31</td>
</tr>
<tr>
<td>7/F/39</td>
<td>No</td>
<td>3.23</td>
</tr>
<tr>
<td>8/F/23</td>
<td>No</td>
<td>5.61</td>
</tr>
<tr>
<td>9/M/53</td>
<td>Yes</td>
<td>8.17</td>
</tr>
</tbody>
</table>

### Table 3. Changes in Urinary 8-Hydroxy-2'-Deoxyguanosine (8-OHdG) Levels, Clinical Activity Scores, and Ophthalmopathy Indexes Among Patients Having Active Graves Ophthalmopathy

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before Treatment</th>
<th>During Treatment</th>
<th>After Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-OHdG level, ng/mg of creatinine</td>
<td>17.47 ± 5.94</td>
<td>7.19 ± 2.91</td>
<td>10.18 ± 4.27</td>
</tr>
<tr>
<td>Clinical activity score</td>
<td>4.88 ± 0.99</td>
<td>2.25 ± 1.04</td>
<td>1.86 ± 0.69</td>
</tr>
<tr>
<td>Ophthalmopathy index</td>
<td>5.50 ± 1.60</td>
<td>3.00 ± 1.20</td>
<td>2.43 ± 0.98</td>
</tr>
</tbody>
</table>

*Data are given as mean ± SD.*

*P<.05 compared with before treatment.*
corticosteroid therapy. Therefore, it is rational to propose that ongoing orbital inflammation triggered changes in the levels of ROS, which caused subsequent free radical-mediated oxidative stress in these patients. Bednarek et al reported that intensive corticosteroid therapy resulted in normalization of peripheral markers for ROS metabolism and restoration of these markers, along with activation of antioxidant defense systems after withdrawal of corticosteroid therapy. In our study, we demonstrated a similar reduction in oxidative DNA damage after systemic corticosteroid treatment. After withdrawal of corticosteroid therapy, the mean urinary 8-OHdG level was statistically significantly lower than that before therapy but was slightly increased compared with that during intensive corticosteroid therapy. This indicates an imbalance between the elevated ROS level and the antioxidant capacity after withdrawal of corticosteroid therapy. In a small case series, the use of oral antioxidants showed encouraging results in the treatment of mild and moderately severe GO. Therefore, based on the results of the present work and previous findings, antioxidant supplementation may be potentially beneficial for these patients after withdrawal of corticosteroid therapy.

Apart from GO, other factors might have been responsible for the increased levels of 8-OHdG. Among other factors, cigarette smoking, which is considered to be the most important known environmental factor associated with GO occurrence and morbidity, may enhance the generation of ROS and reduce the endogenous levels of antioxidants. Patient 3 in our study (Table 1), who had recurrent active GO during tapering of corticosteroid therapy, is a smoker, and his urinary 8-OHdG level was elevated almost to the previous level while receiving systemic corticosteroid therapy. The other smoker with GO (patient 1) had a urinary 8-OHdG level that was more than 4-fold higher than the mean 8-OHdG level of the controls. This suggests that smoking may have a role in the oxidative stress of patients having GO, at least in perpetuating ongoing oxidative damage.

The natural history of GO often includes an initial active phase of progressive exacerbation, followed by regression to a static and inactive phase with residual morbidity of the disease. In this regard, management of GO should rely on the assessment of 2 different features, namely, the severity and activity of the disease. Many indicators of disease severity and activity have been proposed; it is possible that a combination of different variables may better define and characterize the disease condition. In the present study, not only the urinary 8-OHdG level but also the CAS and OI were improved during and after systemic corticosteroid treatment. In addition, the urinary 8-OHdG levels were subsequently elevated in 2 patients having recurrent active GO when corticosteroid therapy was tapered or withdrawn, suggesting that the urinary 8-OHdG level can be used as a valuable variable in the assessment of GO and may potentially help clinicians decide whether a patient requires treatment.

Based on the findings of the present work and previous studies, oxidative stress represents an important pathogenic factor in GO, at least in perpetuating ongoing reactions. However, because of limited cases in this study, more studies are warranted to provide additional information about the precise effect of 8-OHdG level on the natural history of the disease.

In conclusion, we demonstrated that the urinary 8-OHdG level is increased in patients having active GO and that successful management of GO with corticosteroids is associated with a decrease in this oxidative stress marker. The 8-OHdG level in urine is not only a non-invasive biomarker of oxidative stress in patients having GO but also an objective and quantitative variable for the follow-up of immunosuppressive treatment of the disease.

Submitted for Publication: May 31, 2007; final revision received July 21, 2007; accepted July 27, 2007.

Correspondence: Yau-Huei Wei, PhD, Department of Biochemistry and Molecular Biology, National Yang-Ming University, 155 Li-Nong St, Section 2, Taipei 112, Taiwan (joeman@ym.edu.tw).

Financial Disclosure: None reported.

Funding/Support: This study was supported by grant 95-2314-B-075-058 from the National Science Council of Taiwan (Dr Tsai) and by grant V96-B1-002 from Taipei Veterans General Hospital (Dr Tsai).

Additional Contributions: Shi-Bei Wu, MS, and Chun-Yi Liu, MS, at the Department of Biochemistry and Molecular Biology, National Yang-Ming University, provided technical assistance.

REFERENCES

15. Kasai H. Analysis of a form of oxidative DNA damage, 8-hydroxy-2'-deoxyguanosine,

From the Archives of the Archives

The operation presented no difficulties, and, thanks to the circular saw, only occupied 35 minutes from beginning to end. Compared with the usual method of trying to get access from the front, and working in a narrow pocket continually filling with blood, the advantages of Kronlein’s operation are enormous and obvious. In this case under any other method it would have been necessary to sacrifice the eye... Microscopically it presents the characters of a round-celled sarcoma of an aberrant form.


©2007 American Medical Association. All rights reserved.